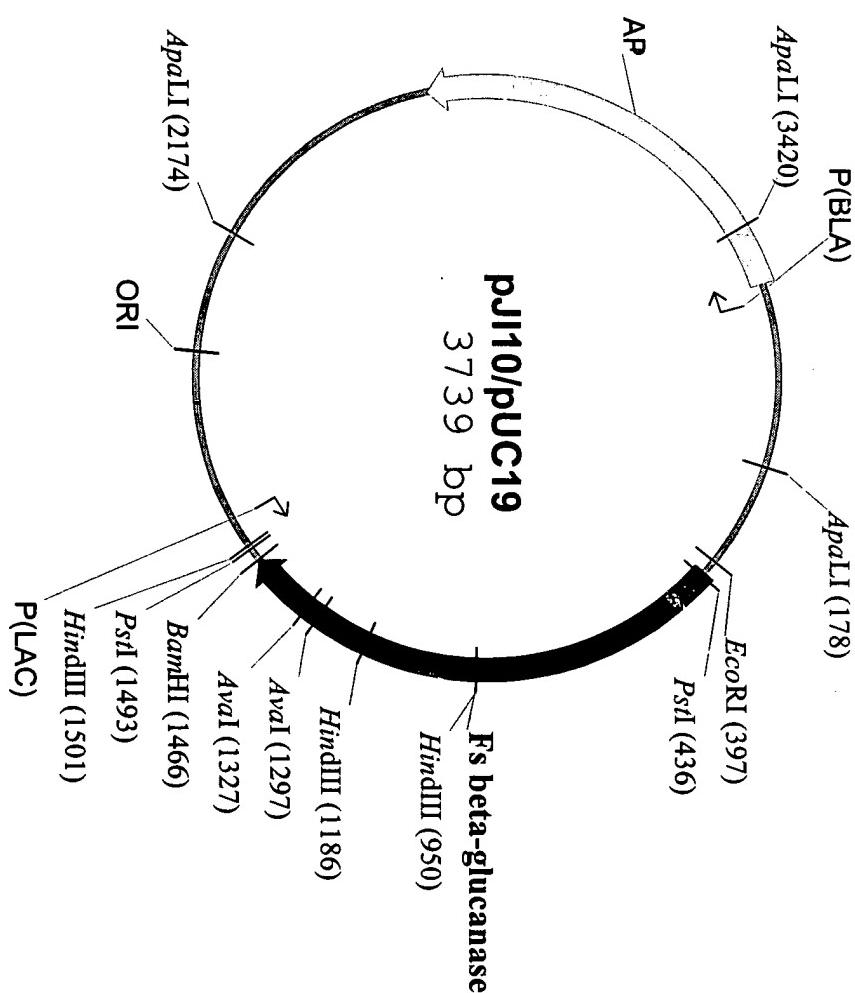


Fig. 1



*Fig. 2 The amino acid sequence in Fig. 1's SEQ ID NO: 1
the DNA sequence in Fig. 2 is SEQ ID NO: 4*

ATGGTTAGCGCAAAGGATTAGCGGTGCCGAACCTACACGTTAGAAGAACAGTCAGTAC	20
M V S A K D F S G A E L Y T L E E V Q Y	
GGTAAGTTGAAGCCGTATGAAGATGGCAGCCGCATGGGAACAGTCAGTCCATGTTG	40
G K F E A R M K M A A A S G T V S S M F	
CTCTACCAGAATGGTCCGAAATGCCGATGGAAGGCCCTGGTAGAAGTGGATATTGAA	60
L Y Q N G S E I A D G R P W V E V D I E	
GTTCTCGGCAAGAACCGGGCAGTTCCAGTCCAACATCATTACCGTAAGGCCGGCGCA	80
V L G K N P G S F Q S N I I T G K A G A	
CAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGATCAGGCTTCCACACC	100
Q K T S E K H H A V S P A A D Q A F H T	
TACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAGGAAGTCCGC	120
Y G L E W T P N Y V R W T V D G Q E V R	
AAGACGGAAGGTGCCAGGTTCCAACTTGACAGGTACACAGGGACTCCGTTAACCTT	140
K T E G G Q V S N L T G T Q G L R F N L	
TGGTCGTCTGAGAGTGCGGCTTGGGTGGCCAGTCGATGAATCAAAGCTTCCGCTTTTC	160
W S S E S A A W V G Q F D E S K L P L F	
CAGTTCATCAACTGGTCAAGGTTATAAGTATACGCCGGCCAGGGCGAAGGCCGGCAGC	180
Q F I N W V K V Y K Y T P G Q G E G G S	
GACTTTACGCTTGACTGGACCGACAATTTGACACGTTGATGGCTCCGCTGGGCAAG	200
D F T L D W T D N F D T F D G S R W G K	
GGTGAATGGACATTGACGGTAACCGTGTGACCTCACCGACAAGAACATCTACTCCAGA	220
G D W T F D G N R V D L T D K N I Y S R	
GATGGCATGTTGATCCTCGCCCTCACCCGCAAAGGTCAAGGAAAGCTTCAACGCCAGGTT	240
D G M L I L A L T R K G Q E S F N G Q V	
CCGAGAGATGACGAAACCTGCTCCG	
P R D D E P A P	248

Fig. 3

The amino acid sequence in Fig. 3 is SEQ ID NO: 2

The DNA sequence in Fig. 3 is SEQ ID NO: 5

ATGGTTAGCGCAAAGGATTAGCGGTGCCGAACCTCACACGTTAGAAGAAGTTCACTAC
 M V S A K D F S G A E L Y T L E E V Q Y 20

GGTAAGTTGAAGCCCGTATGAAGATGGCAGCGCATCGGAACAGTCAGTCCATGTT
 G K F E A R M K M A A A S G T V S S M F 40

CCTTACCAAGAATGGTCCGAAATCGCCGATGGAAGGCCCTGGTAGAACGATGGATATTGAA
 L Y Q N G S E I A D G R P W V E V D I E 60

GTTCTCGGCAAGAATCCGGCAGTTCCAGTCCAACATCATTACCGTAAGGCCGGCA
 V L G K N P G S F Q S N I I T G K A G A 80

CAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGATCAGGCTTCCACACC
 Q K T S E K H H A V S P A A D Q A F H T 100

TACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAGGAAGTCCGC
 Y G L E W T P N Y V R W T V D G Q E V R 120

AAGACGGAAGGTGCCAGGTTCCAACCTGACAGGTACACAGGGACTCCGTTAACCTT
 K T E G G Q V S N L T G T Q G L R F N L 140

TGGTCGTCTGAGAGTGCGGCTTGGTTGCCAGTTCGATGAATCAAAGCTCCGTTTC
 W S S E S A A W V G Q F D E S K L P L F 160

CAGTTCATCAACTGGTCAAGGTTATAAGTATAACGCCGCCAGGGCGAAGGCCAGC
 Q F I N W V K V Y K Y T P G Q G E G G S 180

GACTTACGCTTACTGGACACAATTGACACGTTGATGGCTCCGCTGGGCAAG
 D F T L D W T D N F D T F D G S R W G K 200

GGTGAAGTGGACATTGACGGTAACCGTGTGACCTCACCGACAAGAACATCTACTCCAGA
 G D W T F D G N R V D L T D K N I Y S R 220

GATGGCATGTTGATCCTCGCCCTCACCGCAAAGGTCAAGGAAAGCTCAACGCCAGGTT
 D G M L I L A L T R K G Q E S F N G Q V 240

CCGAGAGATGACGAACCTGCTCCGATTGAGCTCCGTCGACAAGCTTGCAGGCCACTC
 P R D D E P A P N S S S V D K L A A A L 260

GAGCACCACCAACCACCACTGA
 E H H H H H H * |

Fig. 4.

Table 1. Comparison of kinetic properties of *F. succinogenes* and *B. subtilis* 1,3-1,4- β -D-glucanases

Enzyme	Specific activity (U/mg)	k_{cat} (s^{-1})	Opt. Temperature (°C)	Opt. pH
Wild-type	2065 ± 82	1296 ± 51	50 (at pH 6.0)	6.0-8.0
TG-Glucanase	7980 ± 341	3695 ± 158	50 (at pH 6.0)	6.0-8.0
PCR-TF-Glucanase	7833 ± 334	3911 ± 166	50 (at pH 6.0)	6.0-8.0
Lichenase (Megazyme)	118 ^a	47.2 ^a	60 (at pH 6.5) ^a	6.5-7.0 ^a
	82.6 ± 0.96	33.0 ± 0.38	55 (at pH 7.0)	

The kinetics was performed with lichenan (6mg/mL) as substrate in 50 mM citrate buffer (pH 6.0) or in 50mM phosphate buffer (pH 7.0), and at optimum temperature as indicated.

^a: Data was taken from Megazyme instruction brochure of lichenase. The kinetics was done with barley β -glucan (5mg/mL) as substrate.

Fig. 5

Table 3. Reactivation of PCR-TE-glucanase at 25 °C after heat treatment

Heat treatment	Recovery time (min)	Relative activity (%)
90 °C, 10 min	10	68
	20	81
90 °C, 30 min	10	61
	20	67
100 °C, 10 min	10	68
	20	72
100 °C, 30 min	10	55
	20	56

Fig.6a The amino acid sequence in Fig.6 is SEQ ID NO:3
(part a) The DNA sequence in Fig.6 is SEQ ID NO:6

ATGAAACATCAAGAAA	CTGCAGTCAAGAGCGC	TCTGCCGTAGCAGCCG	CAGCAGCAGCC		
M N I K K T A V K S A L A V A A A A A A A A					20
CTCACCAATGTTAGCGCAA	AGGATTAGCGGTGCCG	AACACTCACACGTTAGAAGAA			
L T T N V S A K D F S G A E L Y T L E E					40
GTTCAGTACGGTAAGTTGAAGCCC	TATGAAGATGGCAGCCG	CATCGGGAACAGTCAGT			
V Q Y G K F E A R M K M A A A A S G T V S					60
TCCATGTTCTACCA	AGAATGGTCCGAAATCGCC	GATGGAAGGCCCTGGGTAGAAGTG			
S M F L Y Q N G S E I A D G R P W V E V					80
GATATTGAAGTCTCGGCAAGA	ATCCGGCAGTTCCAGTCCA	ACATCATTACCGGTAAG			
D I E V L G K N P G S F Q S N I I T G K					100
GCCGGCGCACAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGCGATCAGGCT					
A G A Q K T S E K H H A V S P A A D Q A					120
TTCCACACCTACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAG					
F H T Y G L E W T P N Y V R W T V D G Q					140
GAAGTCGCAAGACGGAAGGTGCCAGGTTCCA	ACTTGACAGGTACACAGGGACTCCGT				
E V R K T E G G Q V S N L T G T Q G L R					160
TTAACCTTGTCGTCGAGAGTGCGGCTTGGCCAGTTCGATGAATCAAAGCTT					
F N L W S S E S A A W V G Q F D E S K L					180
CCGTTTCCAGTCATCAACTGGGTCAAGGTTATAAGTATA	CGCCGGGCCAGGGCGAA				
P L F Q F I N W V K V Y K Y T P G Q G E					200
GGCGGCAGCGACTTACGCTTGACTGGACCGACA	ATTGACACGTTGATGGCTCCCGC				
G G S D F T L D W T D N F D T F D G S R					220
TGGGGCAAGGGTGA	CTGGACATTGACGGTAACCGTGT	CGACCTCACCGACAAGAACATC			
W G K G D W T F D G N R V D L T D K N I					240
TACTCCAGAGATGGCATGTTGATCCTCGCCCTCACCGCA	AAAGGTCAAGGAAAGCTCAAC				
Y S R D G M L I L A L T R K G Q E S F N					260
GGCCAGGTTCCGAGAGATGACGAACCTGCTCCGCA	ATCTTAGCAGCGCTCCGGC	CATCT			
G Q V P R D D E P A P Q S S S S A P A S					280
TCTAGCAGTGTCCGGCAAGCTCCTCTAGCGTCCCTGC	CCTCGAGCAGCGCATTGTT				
S S S V P A S S S S V P A S S S S A F V					300
CCGCCGAGCTCCTCGAGCGCCACAAACGCA	ATCCACGGAATGCGCACA	ACTCCGGCAGTT			

Fig.
(part b)

P P S S S A T N A I H G M R T T P A V 320
GCAAAGGAACACCGCAATCTCGTGAACGCCAAGGGTCCAAGGTGAACCCGAATGGCCAC
A K E H R N L V N A K G A K V N P N G H 340
AAGCGTTATCGCGTGAACCTTGAAACACTAA
K R Y R V N F E H * 349

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